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Exploring the Antibacterial Potential of Essential Oils Extracted from Three Medicinal Plants Against Some Foodborne Bacteria

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ABSTRACT

This study aims to evaluate the antibacterial activity of essential oils extracted from the green leaves of three medicinal plants, namely *Cupressus macrocarpa, Schinus terebinthifolius*, and *Eucalyptus citriodora*, against selected foodborne bacteria. Gas chromatography/mass spectrometry (GC/MS) analysis was employed to identify the chemical composition of the extracted essential oils. The two main chemical components of *C. macrocarpa* essential oils (EO) were terpinene-4-ol (32.37%) and citronellol (29.29%). The primary components of *S. terebinthifolius* EO were α-phellandrene (44.35%) and o-cymene (10.42%). Meanwhile, α-phellandrene (13.5%) and sabinene (24.24%) constitute the majority of *E. citriodora* EO. The antibacterial activity of the essential oils was assessed against Gram-positive bacteria including *Bacillus cereus, Staphylococcus saprophyticus, Lysinibacillus fusiformis*, and *Kocuria rhizophila*, as well as the Gram-negative bacterium *Serratia liquefaciens*. Standard antibiotics like Penicillin, Amoxicillin, and Ampicillin were used for comparison. The results revealed varying degrees of antibacterial activity against each pathogenic isolate used. *C. macrocarpa* essential oil exhibited the strongest antibacterial action, with a 55.7 mm inhibition zone diameter against *Bacillus cereus*, which displayed resistance to the tested standard antibiotics. Meanwhile, essential oils of the choosed plants also inhibited the growth of *S. liquefaciens* despite its tolerance to the tested antibiotics. The minimum inhibitory concentrations (MIC) of *C. macrocarpa*, *S. terebinthifolius*, and *E. citriodora* essential oils ranged from 0.06 to 1.5 mg/ml, 0.68 to 2.0 mg/ml, and 0.2 to 1.77 mg/ml, respectively. These findings highlight the potential of the tested essential oils as antibacterial agents for preserving food materials in a safe, sustainable, cost-effective, and eco-friendly manner.

Keywords: Antibacterial activity, foodborne bacteria, Food preservation; Essential oils, Medicinal plants.

INTRODUCTION

Essential oils (EO) derived from medicinal plants are valuable secondary metabolites widely utilized in the food industry for their fragrance and antimicrobial properties (Serag *et al.*, 2022). Owing to their natural origin and inherent protective properties in plants, essential oils have garnered significant interest as potential antimicrobial agents (Gunasena *et al.*, 2022). These oils are composed of aromatic volatile compounds obtained from various parts of plants (Wang *et al.*, 2020). Microbial contamination poses a hidden risk to food safety, making the exploration of effective antimicrobial strategies crucial.

The utilization of synthetic antimicrobial agents has proven to be an effective approach in inhibiting microbial growth, reducing the risk of food poisoning, and extending the shelf life of food products (Shatalov *et al.*, 2017). The food industry faces significant challenges due to the contamination of food with pathogenic microorganisms, such as bacteria and fungi, which produce toxins that degrade food quality during pre- and postharvest processing (Maurya *et al*., 2021). To address this issue, chemical preservatives have been employed, including formaldehyde, sodium benzoate, pyrrolidines, sulfites, sodium nitrite, sulfur dioxide, benzoates, imidazoles, thiocyanates, and sorbates, demonstrating their efficacy in reducing microbial contamination in food (Gutiérrez-del-Río *et al*., 2018).

In light of their potential human health risks, including carcinogenesis, teratogenesis, environmental toxicology, and long-term degradation (Falleh *et al*., 2020), the use of chemical preservatives mentioned earlier raises concerns about negative health impacts. In addition, these synthetic preservatives can also accumulate in the environment, leading to pollution and potential harm to ecosystems (Falleh *et al*., 2020). Furthermore, the emergence of antibiotic-resistant foodborne pathogens poses a significant threat to public health and food security. The misuse or overuse of antibiotics in both human medicine and agriculture has contributed to the development of antibiotic resistance among bacterial pathogens (Bhatia *et al*., 2021). This has led to the need for alternative strategies to combat these pathogens and ensure the safety of our food supply. Therefore, the development of natural preservatives has emerged as an alternative approach (Marrone *et al.*, 2021). To combat this issue, numerous studies have highlighted the potent antibacterial properties of plant essential oils (Iseppi *et al*., 2019). These natural alternatives show promise in overcoming the challenges associated with synthetic antimicrobial agents and chemical preservatives, contributing to the safeguarding of food safety and public health.

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The utilization of natural plant extracts in the field of food preservation has gained significant attention. *Cupressus macrocarpa*, a medicinal plant belonging to the Cupressaceae family, possesses various pharmacological properties such as hepatoprotective, anti-inflammatory, and antiviral activities (Al-Sayed *et al*., 2018; Negm *et al*., 2020). The essential oil derived from *C. macrocarpa* has been found to exhibit potent antibacterial and antifungal properties, effectively targeting a wide range of bacteria and fungi (Attallah *et al*., 2021). Another plant of interest is *Schinus terebinthifolius* Raddi, a perennial woody plant from the Anacardiaceae family (Belhoussaine *et al*., 2022). This plant has been associated with various pharmacological activities, including anti-inflammatory effects (Silva *et al*., 2017), antimicrobial properties (da Silva *et al*., 2018), and antioxidant activity (Rocha *et al*., 2018). *Eucalyptus citriodora* Hook, a member of the Myrtaceae family (Valenzuela *et al.*, 2021), has also demonstrated diverse activities, including antibacterial, antifungal, and pharmaceutical properties (Gu *et al*., 2019). Such characteristics make *E. citriodora* a promising candidate for natural preservation methods in the food industry. The exploration of these medicinal plants and their essential oils provides potential alternatives to synthetic antimicrobial agents and chemical preservatives. By harnessing the antibacterial and antifungal properties of *Cupressus macrocarpa*, *Schinus terebinthifolius*, and *Eucalyptus citriodora*, researchers aim to develop natural preservatives that can effectively inhibit microbial growth, reduce food contamination, and preserve food quality.

Regarding foodborne pathogens, *S. saprophyticus* is a common contaminant found in meat, poultry-contaminant and fermented foods, as well as in the intestinal and rectal flora of animals like pigs and cattle (Becker *et al*., 2014). This bacterium is notorious for causing urinary tract infections, primarily by colonizing the gastrointestinal tract and subsequently being inadvertently transferred to the urethra through contaminated feces (Sommers *et al*., 2017). Another significant foodborne pathogen is *Bacillus cereus*, which has been associated with outbreaks worldwide (Elafify *et al*., 2023). Meanwhile, *Lysinibacillus fusiformis*, a rod-shaped Gram-positive bacterium, poses a threat to human health, causing tropical ulcers, severe sepsis, and respiratory illnesses (Sulaiman *et al.*, 2018). Additionally, *Kocuria* organisms, belonging to the Gram-positive coccoid bacteria group in the Micrococcaceae family, can cause various infections, especially in individuals with compromised immune systems and underlying disorders (Savini *et al*., 2010). Notably, the recent reclassification of the ATCC 9341 strain as *Kocuria rhizophila* from *Micrococcus luteus* adds to our understanding (Tang *et al*., 2003). Finally, *Serratia*, a Gram-negative member of the Enterobacteriaceae family, is recognized for its association with food spoilage. *S. liquefaciens*, a species within this genus, acts as an opportunistic pathogen in humans and is highly pathogenic to humans, insects, and fish (Mahlen, 2011).

The infections caused by *Serratia* species can be severe, even leading to fatality (Samonis *et al*., 2011; Parte *et al*., 2020). Given the risks posed by these foodborne pathogens, the development of natural preservatives becomes crucial. Therefore, this study aims to investigate the potential activity of essential oils of *Cupressus macrocarpa, Schinus terebinthifolius*, and *Eucalyptus citriodora* as antibacterial against some foodborne bacteria.

MATERIALS AND METHODS

Plant material collection

Fresh green leaves of *Cupressus macrocarpa*, *Schinus terebinthifolius*, and *Eucalyptus citriodora* were collected from the Botanical Garden of the Faculty of Education, Damietta University, during the Spring-Summer season of 2021. The collected plant samples underwent authentication by Dr. Mamdouh S. Serag, a Professor of Plant Ecology in the Botany and Microbiology Department of the Faculty of Science, Damietta University. To ensure proper documentation and future reference, plant specimens were carefully prepared and deposited in the herbarium of the Botany and Microbiology Department, Faculty of Science, Damietta University. This repository serves as a valuable resource for the verification and further study of the collected plant samples.

Extraction of essential oils

Fresh leaves of the selected plants (500 g) were washed with distilled water, segmented into small parts, then added distilled water and heated to boiling using a Clevenger-type apparatus and hydro-distillation for 5 hours**.** After boiling, the essential oil evaporated, condensed as the upper phase, and separated from the lower one. The extracted oil was dried over anhydrous sodium sulfate and kept in a closed, dark glass vial at 4 ℃ in the refrigerator until analysis (Boukhris *et al.*, 2012).

Gas chromatography-Mass spectrometry analysis (GC/MS)

At the Central Laboratories Network, National Research Centre, Cairo, Egypt, a gas chromatograph (789-0B) and mass spectrometer detector (5977A) were part of the GC-MS system (Agilent Technologies). An HP-1MS column (60 m x 0.25 mm internal diameter and 0.25 µm film thickness) was installed in the GC. Helium was used as the carrier gas for the analyses, with a flow rate of 1 ml/min, a split ratio of 30:1, an injection volume of 0.5 µl, and a temperature program of 40 °C for 1 min, followed by a rise of 5 °C/min to 200 °C. The injector and detector were kept at respective temperatures of 250 and 280 °C. By employing an m/z range of 50-550 and a solvent delay of five minutes, mass spectra were produced by electron ionization (EI) at 70 eV. The spectrum fragmentation pattern was compared to those stored in the Wiley and NIST Mass Spectral Library data to identify various constituents.

Media used for isolation

Different media were utilized for the isolation of foodborne bacteria, including nutrient agar and Nutrient broth. Nutrient agar, comprising 5.0g peptone, 3.0g beef extract, 8.0g sodium chloride, and 12.0g agar per liter, was purchased from Neogen (Heywood, UK)

for solid medium preparation (Atlas, 2010). Nutrient broth, containing 5.0g peptone, 3.0g beef extract, 5.0g sodium chloride, 5.0g glucose, and distilled water to achieve a final volume of 1000 ml, was also obtained (Atlas, 2010). Additionally, Mueller Hinton Agar medium, purchased from Britania SA Labs (Buenos Aires, Argentina), was also used, consisting of (g/L): 3.0 beef extract, 17.5 acid casein hydrolysate, 1.5 starches, and 15.0 agar. These media were selected based on their established formulations and suitability for the Isolation and cultivation of foodborne bacteria (Atlas, 2010).

Isolation of bacterial strains

All the tested bacteria were isolated from spoiled food samples, specifically beef burgers, beef luncheons, and sausage, in the Microbiology laboratory at the Faculty of Science, Damietta University. The samples were randomly purchased from various markets in New Damietta, Egypt, and were immediately transferred to sterile polyethylene bags to maintain aseptic conditions. These samples were then stored at room temperature to create favorable conditions for spoilage and microbial growth.

Morphological, biochemical, and molecular techniques for Identification of Isolated Bacterial Strains

The isolated bacterial strains were subjected to a comprehensive identification process involving morphological, biochemical, and molecular techniques. Morphological characteristics, such as colony morphology and cell shape, were observed under a microscope. Additionally, Gram staining (McClelland, 2001) and spore staining (Gerhardt, 1994) were performed for further aid in the identification process. The strains were assessed based on their shape, pigment production, elevation, edge, color, and opacity.

Biochemical tests were conducted to gather additional information about the bacterial strains. Carbohydrate fermentation tests and enzyme activity assays were employed to determine the metabolic properties of the isolates. These tests provided valuable insights into the metabolic capabilities of the strains, assisting in the characterization and identification process. In order to achieve higher accuracy and specificity in identification, molecular technique utilizing 16S rRNA was employed. Polymerase chain reaction (PCR) was utilized to amplify the 16S rRNA gene, and subsequent DNA sequencing was performed. This molecular approach allowed for more precise identification of the bacterial isolates. Overall, a combination of morphological, biochemical, and molecular methods was employed to comprehensively identify the isolated bacterial strains.

Biochemical characteristics

Bacterial isolates were identified using Bergey's manual of determinative bacteriology (Breed *et al.*, 1957; Grimont and Grimont, 2006; and Langlois *et al.*, 1990). Biochemical tests including catalase (Cappucino and Sherman, 2001), starch hydrolysis, Voges Proskauer, urea hydrolysis, citrate utilization, Hydrogen sulfide (H_2S) production (Atlas, 2010), Sugar fermentation test (MacFaddin, 2000), indole production (Ojokoh and Eromosele, 2015), tube coagulase test (katz *et al.*, 2010), oxidase test (MacFaddin, 2000), and esculin hydrolysis (Atlas, 2010).

Molecular identification of the isolated bacterial strains

DNA extraction

According to the manufacturer's instructions, genomic DNA was extracted and purified from bacterial samples using ABT DNA mini extraction kit (Applied Biotechnology Co. Ltd, Egypt).

PCR amplification of 16S rRNA

PCR was carried out using the universal primer pair 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). PCR reactions were performed in a volume of 50 µl (2x Red Master mix, 20 picomoles of each primer, and 100 ng of genomic DNA) using a thermal cycler (MJ Research, USA). PCR cycling conditions were as follows: one cycle of initial denaturation stage at 95 ºC for 3 min, followed by 35 amplification cycles [Denaturation at 95ºC for 30 s, annealing at 50 ºC for 30 s, extension at 72 ºC for 90 s], then a final extension step at 72 ºC for 5 min. PCR-DNA products (5µl) were visualized on ethidium bromide-stained 1% agarose gel (w/v) in Tris-acetate EDTA (TAE) buffer by a gel electrophoresis system (Shokr *et al.*, 2023). Electrophoresis was performed at a constant 80V for 30min. The amplified product was visualized as a single compact band of expected size under UV light and documented by the Samsung Note4 smartphone.

DNA sequencing for the amplified genes

The amplified PCR products were sent to Solgent Co Ltd (South Korea) for gel purification and sequencing. The amplified PCR products amplified by the universal primer were sequenced using forward and reverse primers. Sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing Kits and model ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Sequence alignment and phylogenetic analysis

The resulting sequences were quality trimmed and assembled in Geneious software; consequently, the trimmed sequences were identified by search in the basic local alignment Nucleotide Blast tool (BLASTn) of the GenBank database (http://www.ncbi.nlm.nih.gov.blast). Nucleotide sequences obtained, together with corresponding sequences retrieved from the GenBank, were aligned using MAFFT alignment (Katoh and Standley, 2013). Phylogenetic trees were constructed using MEGA-X software and the Neighbor-joining method (Saitou and Nei, 1987), employing the Tamura-Nei Model (Tamura and Nei, 1993). The trees were assessed using 1000 bootstrap replicates.

Inoculum preparation

Bacterial cultures stocks were streaked on nutrient agar medium and incubated for 24 h at 37 °C, then one colony of each isolate was inoculated in 5ml nutrient broth in sterile test tubes for 16h, at 37 °C in a shaking incubator (Model: PT. DAIHAN LABTECH) with 150 rpm. 1ml was transferred to 10 ml of sterile nutrient broth medium. After incubation at 37°C until the cultures reached the mid-exponential growth phase:(OD600 = 0.5), the cultures were diluted with

sterile physiological saline solution (0.85%, sodium chloride) until the turbidity of each suspension was adjusted to match 0.5 McFarland standard (OD600 = 0.132), corresponding to a bacterial density of about 10^8 CFU/ml (Close *et al.*, 2012).

Essential oil emulsion preparation

Tween 80 (1% v/v) was used as an emulsifier for preparing emulsions of the essential oils in nutrient broth medium with a concentration of 55 µl/ml. Tween 80 was sterilized using 0.22µm Millipore filter (Kpadonou *et al*., 2022).

Antimicrobial activity test

The antimicrobial activity of the essential oils (EO) derived from the selected plants was assessed using the agar well diffusion technique. In this method, 0.2 ml of each bacterial suspension was inoculated onto Mueller-Hinton agar medium (Britania) and poured into Petri dishes. Wells with a diameter of 9 mm were created using a sterile cork borer, and 150 µl of undiluted oil was added to each well. As controls, Penicillin (10 µg/disk), Ampicillin (10 µg/disk), and Amoxicillin (25 µg/disk) were used. The diameter of the inhibition zones was measured after incubation for 24 hours at 37°C (Balouiri *et al*., 2016). This assay was conducted in triplicate, and the mean value of the results was calculated and reported.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) assay was conducted to determine the lowest concentration of the essential oil required to inhibit the growth of the tested bacteria. Initially, 1 ml emulsion of the essential oil was prepared. Serial dilutions of the emulsion were then performed in nutrient broth (NB) medium using sterile test tubes, resulting in final concentrations ranging from 0.075 to 18.33 µl/ml. To each diluted sample, a bacterial suspension with a final concentration of 10^7 CFU/ml was added. The samples were incubated at 37°C for 24 hours in a shaking incubator set at 150 rpm (Huang *et al*., 2021). In this assay, control groups were included. These controls consisted of culture medium (NB) only, culture medium (NB) with the bacterial suspension, and NB containing 1% of tween 80 along with the bacterial suspension. To assess bacterial growth, turbidity was measured compared to the control turbidity using a spectrophotometer (Unico, model 7200, made in the USA) at a wavelength of 600 nm (D'Aquila *et al*., 2022). The MIC was determined as the lowest concentration of the essential oil at which no visible growth (turbidity) of the tested bacteria was observed (Huang *et al*., 2021). This assay was performed in triplicate to ensure the reliability of the results.

The potential antibacterial activity of the extracted essential oils

This assay determined the investigated essential oil had bactericidal or bacteriostatic properties on the tested bacteria. When the bacteria failed to grow on Nutrient broth (NB) and failed to grow again on Nutrient agar (NA) (NEOGEN Culture Medium), even after removing the effect of the essential oil and permanently killed, the effect is bactericidal, but when the bacteria failed to grow on NB and return to grow on NA after removing the effect of essential oil and temporarily stopped from growing, it means bacteriostatic (Smith *et al.*, 1998). This test depended on the previous assay MIC. From the tubes where the bacteria failed to grow and were inhibited at MIC concentration, 50µl was removed, cultured on NA, and incubated at 37°C for 24h for the tested bacteria.

Statistical analysis

Results are reported as the mean of three replicates ± standard error (SE). Data were subjected to one-way ANOVA using SPSS (ver. 26). Mean separation was performed using Duncan's multiple ranges at $P<0.05$ (Kleinbaum *et al.*, 2013).

RESULTS

Identification of bacterial isolates

The bacterial isolates were identified using morphological and biochemical characteristics, as shown in Tables (1) which resulted in the identification of two *Bacillus* spp (B1, S1), *Staphylococcus coagulase* negative spp (L1), *Serratia* spp (S3), and *micrococcus* spp (L3).

Molecular identification of the bacterial isolates using 16S rRNA gene analysis

Molecular identification revealed that the bacterial isolates are *Bacillus cereus*, *Staphylococcus saprophyticus*, *Lysinibacillus fusiformis*, *Kocuria rhizophila*, and *Serratia liquefaciens* for the bacterial isolates (B1, L1, S1, L3, and S3, respectively) with similarity of 100% then recorded with accession numbers listed in Table (2). Phylogenetic relationships of the isolated strains are shown in Figures (1), (2), and (3). The neighbor-joining method was employed using the maximum composite likelihood bootstrapping with 1000 replicates.

The water content of the selected plant species

The water content, moisture content, and succulent values of the three selected medicinal plants are presented in Table (3). *Cupressus macrocarpa* displayed the highest water content, with a moisture content of 54.62%. Conversely, *Eucalyptus citriodora* exhibited the lowest water content, with a moisture content of 38.94%. The succulent values ranged from 1.36 to 2.20 across the selected plant species.

GC/MS analysis of the extracted essential oils

Gas chromatography-mass spectrometry (GC/MS) analysis of the medicinal plant tested was employed to identify the constituents of the extracted essential oils. Gas chromatography-mass spectrometry (GC/MS) analysis was performed to identify the constituents of the extracted essential oils from the medicinal plant samples. The results of the GC/MS analysis for each plant are summarized in Tables (4), (5), and (6). Table (4) displays the analysis of *C. macrocarpa* essential oil, revealing the presence of nineteen bioactive compounds, which accounted for 100% of the total oil composition. The major components identified were terpinene-4-ol (32.37%) and citronellol (29.29%), followed by isopulegol (7.06%) and camphor (6.21%) (Fig.4A). However, in the essential oil of *S. terebinthifolius*, a total of twenty compounds were identified

Table (1): Morphological and biochemical characteristics used for identification of bacterial Isolates.

Characterization	Tested bacterial isolates				
	L1	**B1**	**S1**	**S3**	**L3**
Macromorphology On NA medium					
Shap	Circular	Circular	Circular	Circular	Circular
Color	White	White	Pale- white	White	yellow
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Margins	Entire	Irregular	Entire	Entire	Entire
Elevation	convex	Low	convex	Raised	convex
Micromorphology					
Shap	Cocci	Rod	Rod	Rod	Cocci
Aggregation	Cluster	Short chain-diplo-mon	Monobacilloid	Mono-Cluster	Mono-Cluster
Ability to Gram stain	+	+	+	-	+
Spore formation	-	+	+	-	-
Pigment Production	-	-	-	-	+
Biochemical Test					
Catalase	+	+	+	+	+
Coagulase	-	ND	ND	**ND**	ND
Voges Proskauer	-	+	-	-	-
Indole production	-	-	-	-	-
Oxidase test	ND	ND	ND	-	ND
Starch hydrolysis	-	+	-	-	-
H_2S production	-	-	-	-	-
Urea hydrolysis	+	+	+	-	-
Citrate utilization	-	+	+	+	-
Esculin hydrolysis	ND	ND	ND	+	ND
Sugar Fermentation					
Mannitol	+	-	-	+	-
Lactose	+	-	-	+	-
Ability to grow on 5% NaCl	+	+	+	+	+

+, positive reaction result; -, negative reaction result; ND, not detected.

Table (2): Molecular Identification of foodborne bacterial isolates, recovered from different food sources, using 16S rRNA sequencing analysis.

Isolate code	Source of isolation	Sequence length (bp)	BLASTn result		Final identification	Accession number
			Closest species	Similarity %		
B1	Beef burger	797	*Bacillus cereus*	100	*Bacillus cereus* DU-BST	OQ380712
L1	Beef luncheon	1380	*Staphylococcus saprophyticus*	100	*Staphylococcus saprophyticus* DU-LA	OQ071703
S1	Sausage	1377	*Lysinibacillus fusiformis*	100	*Lysinibacillus fusiformis* DU-SST	OQ071701
L3	Beef luncheon	1375	*Kocuria rhizophila*	100	*Kocuria rhizophila* DU-Yell	OQ071700
S3	Sausage	738	*Serratia liquefaciens*	100	*Serratia liquefaciens* DU-S3	OQ071699

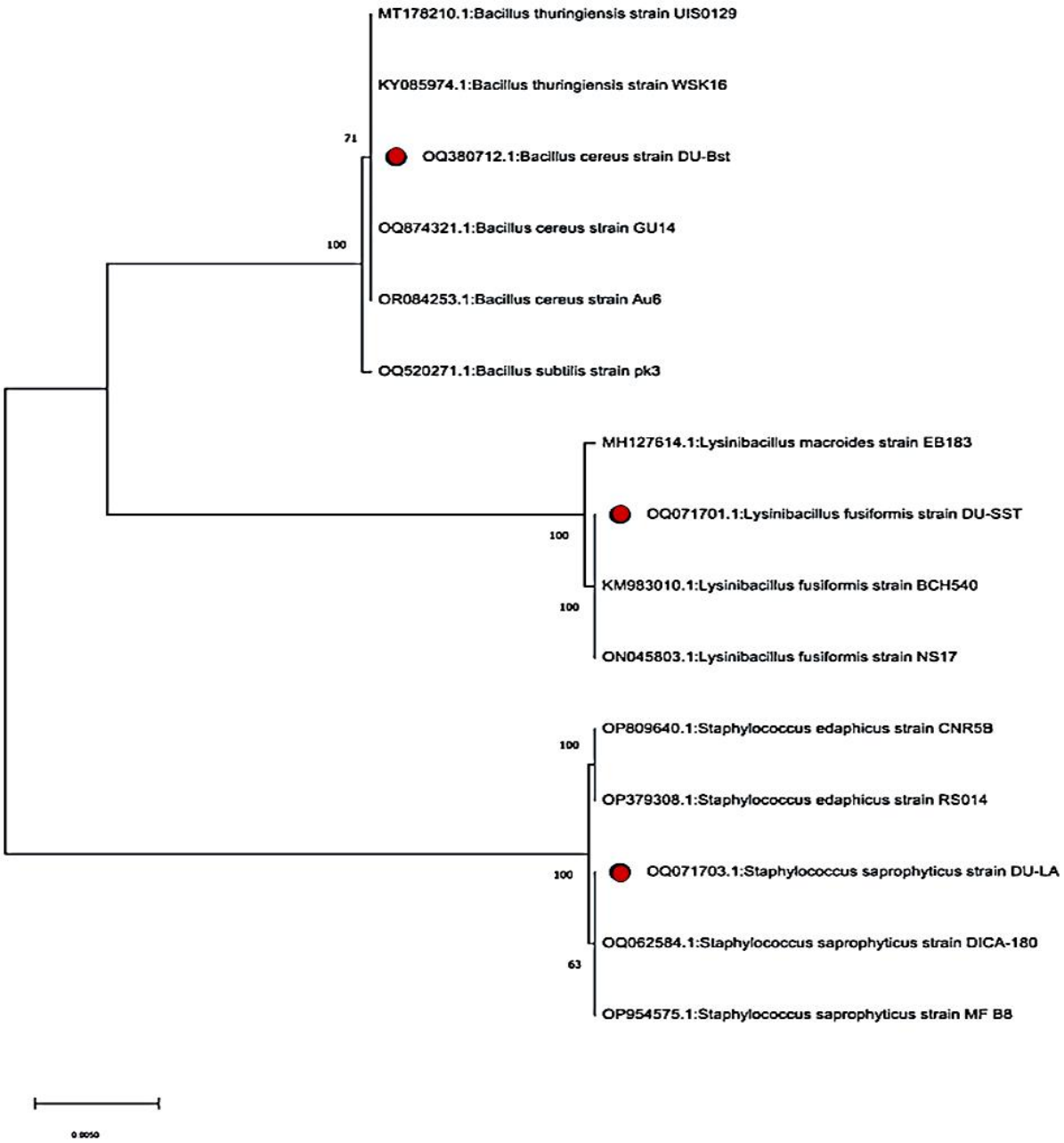

Figure (1): Phylogenetic dendrogram of *B. cereus* (DU-Bst), *Lysinibacillus fusiformis* (DU-SST), and *Staphylococcus saprophyticus* (DU-LA) strains, along with related species, based on 16s rRNA gene sequence alignment. The neighbor-joining was performed using the maximum composite likelihood bootstrapping (1000 replicates).

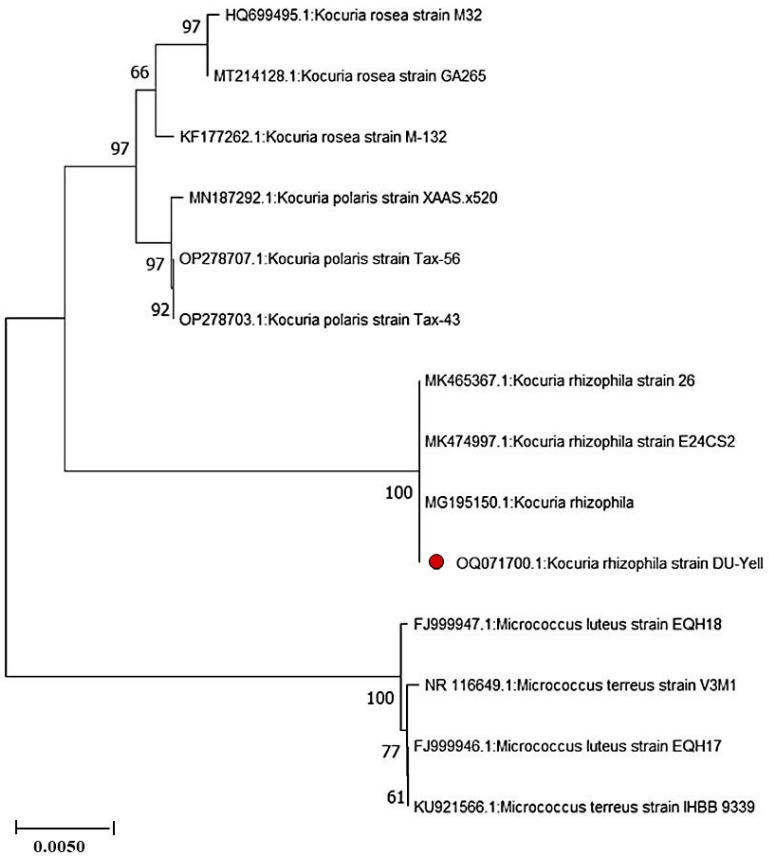

Figure (2): Phylogenetic dendrogram of *K. rhizophila* (DU-yell) strain along with related species based on 16S rRNA gene sequence alignment. The neighbor-joining was performed using the maximum composite likelihood bootstrapping (1000 replicates).

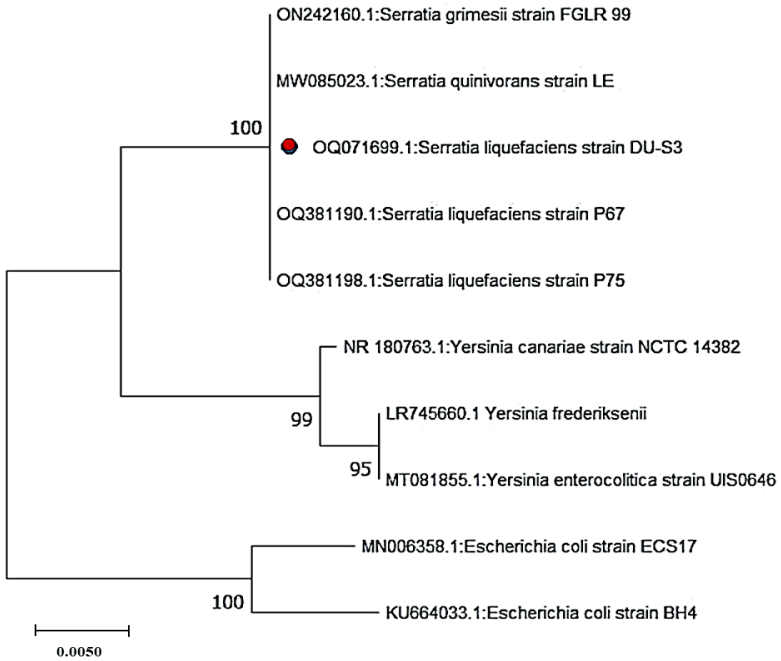

Figure (3): Phylogenetic dendrogram of *S. liquefaciens* (DU-S3) strain along with related species based on 16S rRNA gene sequence alignment. The neighbor-joining was performed using the maximum composite likelihood bootstrapping (1000 replicates).

representing 100% of the total oil (Fig. 4B).

The main component was α-phellandrene, accounting for 44.35% of the composition, followed by o-cymene (10.42%) and limonene (6.44%) (Table 5). On the other hand, *Eucalyptus citriodora* essential oil contained twenty six bioactive compounds, repressenting 100% of the total oil composition (Fig. 4C). The major components in *Eucalyptus citriodora* oil were identified as Sabinene (24.24%), α-phellandrene (13.5%), α-eudesmol (7.03%), Spathulenol (6.41%), and o-Cymene (6.09%) (Table 6).

Antibacterial activity of the essential oils

As presented in Figure (5), the essential oil of *C. macrocarpa* inhibited *B. cereus*, showed the highest inhibition zone of 55.7±1.2 mm, and exhibited resistance to the tested antibiotics compared to other tested bacteria. However, *S. saprophyticus* showed inhibition zones of 22.7±1.2 and 31.97±0.55 mm *L. fusiformis*, 17.5±0.74, and 17.3±0.88 mm for *K. rhizophila*, and *S. liquefaciens*, respectively. *S. terebinthifolius* showed a higher effect against *S. saprophyticus* with a 24.97 ±0.84 mm inhibition zone, followed by *K. rhizophila* and *L. fusiformis* with inhibition zones of 23.4±1.32 and 23.4±0.17 mm, respectively.

B. cereus and *S. liquefaciens* showed less sensitivity for *S. terebinthifolius* as it affected *B. cereus* with an inhibition zone of 13.8 ±0.6 and 13.3 ±0.33 mm for *S. liquefaciens.* For *E. citriodora* EO, *B. cereus* showed the highest inhibition zone of 32.5±0.9 mm,

Table (3): Water content in the selected medicinal plants

Medicinal Plant species tested	Measured parameters		
	Water content (g g^{-1} DM)	Moisture %	Succulent value
C. macrocarpa	8.09	54.62	2.20
S. terebnthifolias	6.62	44.90	1.82
E. citriodora	5.03	38.49	1.63

Table (4): Phytochemical composition of essential oils extracted from *C. macrocarpa* using Gas Chromatography/Mass Spectrometry (GC/MS) Analysis.

Peak	Compound name	Chemical Classification	Retention time (RT, min)	Molecular Formula	Molecular weight (M.wt)	Area %
1	α-thujene	Monoterpene	8.025	$C_{10}H_{16}$	136	0.17
2	sabinene	Monoterpene	9.233	$C_{10}H_{16}$	136	4.31
3	(-)-.β -pinene	Monoterpene	9.776	$C_{10}H_{16}$	136	1.07
4	α- terpinene	Monoterpene	10.486	$C_{10}H_{16}$	136	1.89
5	o-cymene	Monoterpene	10.577	$C_{10}H_{14}$	134	0.76
6	limonene	Monoterpene	10.846	$C_{10}H_{16}$	136	0.89
7	γ -terpinene	Monoterpene	11.687	$C_{10}H_{16}$	136	3.27
8	cis-sabinene hydrate	Oxygenated monoterpene	11.951	$C_{10}H_{18}O$	154	0.88
9	α-terpinolene	Monoterpene	12.557	$C_{10}H_{16}$	136	1.29
10	trans sabinene hydrate	Oxygenated monoterpene	12.832	$C_{10}H_{18}O$	154	1.24
11	linalyl acetate	Oxygenated monoterpene	12.883	$C_{12}H_{20}O_2$	196	2.67
12	2-cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, cis-	Oxygenated monoterpene	13.496	$C_{10}H_{18}O$	154	1.58
13	camphor	Oxygenated monoterpene	13.845	$C_{10}H_{16}O$	152	6.21
14	2-cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, trans-	Oxygenated monoterpene	13.976	$C_{10}H_{18}O$	154	1.13
15	Isopulegol	Oxygenated monoterpene	14.217	$C_{10}H_{18}O$	154	7.06
16	terpinen-4-ol	Oxygenated monoterpene	15.052	$C_{10}H_{18}O$	154	32.37
17	α-terpineol	Oxygenated monoterpene	15.367	$C_{10}H_{18}O$	154	3.22
18	2-cyclohexen-1-ol,3-methyl-6-(1-methylethyl)-, cis-	Oxygenated monoterpene	15.899	$C_{10}H_{18}O$	154	0.70
19	citronellol	Oxygenated monoterpene	16.557	$C_{10}H_{20}O$	156	29.29
Total identified						**100.00**

Table (5): Phytochemical composition of essential oils extracted from *Schinus terebinthifolius* using Gas Chromatography/Mass Spectrometry (GC/MS) Analysis.

Peak No.	Compound name	Chemical Classification	Retention time (RT, min)	Molecular Formula	Molecular wteight (Mwt)	Area %
1	α-pinene	Monoterpene	8.203	$C_{10}H_{16}$	136	4.70
2	(-)-.β -pinene	Monoterpene	9.776	$C_{10}H_{16}$	136	1.28
3	α-phellandrene	Monoterpene	10.125	$C_{10}H_{16}$	136	44.35
4	o-cymene	Monoterpene	10.577	$C_{10}H_{14}$	134	10.42
5	sabinene	Monoterpene	10.789	$C_{10}H_{16}$	136	3.10
6	limonene	Monoterpene	10.846	$C_{10}H_{16}$	136	6.44
7	α- terpinolene	Monoterpene	12.563	$C_{10}H_{16}$	136	1.87
8	terpinen-4-ol	Oxygenated monoterpene	15.035	$C_{10}H_{18}O$	154	1.45
9	α- terpineol	Oxygenated monoterpene	15.372	$C_{10}H_{18}O$	154	0.56
10	citronellol	Oxygenated monoterpene	16.534	$C_{10}H_{20}O$	156	4.61
11	5-isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	Oxygenated monoterpene	16.643	$C_{10}H_{16}O_2$	168	4.78
12	cis-p-mentha-1(7),8-dien-2-ol	Oxygenated monoterpene	18.662	$C_{10}H_{16}O$	152	2.38
13	bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, [1s-(1.alpha., 3.beta.,5.alpha.)]-	Oxygenated monoterpene	20.568	$C_{10}H_{16}O$	152	1.72
14	α-selinene	Sesquiterpene	21.071	$C_{15}H_{24}$	204	1.52
15	caryophyllene	Sesquiterpene	21.77	$C_{15}H_{24}$	204	3.05
16	β- cubebene	Sesquiterpene	23.229	$C_{15}H_{24}$	204	1.79
17	germacrene b	Sesquiterpene	23.606	$C_{15}H_{24}$	204	1.90
18	(-)-β-elemene	Sesquiterpene	23.807	$C_{15}H_{24}$	204	0.67
19	γ-elemene	Sesquiterpene	25.008	$C_{15}H_{24}$	204	2.51
20	(-)-spathulenol	Oxygenated Sesquiterpene	25.357	$C_{15}H_{24}O$	220	0.91
Total identified						**100.00**

followed by *K. rhizophila* 31.9±1.26 mm. *S. saprophyticus*, *L. fusiformis*, and *S. liquefaciens* showed inhibition zones of 25.5 ±0.76, 22.8 ±1.01, and 18.3 ±1.20 mm, respectively. Tested antibiotics like Penicillin (10 µg/disk), Amoxicillin (25µg/disk), and Ampicillin (10 µg/disk) were used as positive control. *B. cereus* showed resistance against all the tested antibiotics. *S. liquefaciens* was found to be sensitive only to Amoxicillin but resistant to Penicillin and Amoxicillin. *K. rhizophila*, *L. fusiformis*, and *S. saprophyticus* showed sensitivity to Amoxicillin and Penicillin but were less sensitive to Ampicillin (Table 7). The essential oils had a very significant ($p \leq 0.001$) effect on antibacterial activity.

Minimum inhibitory concentration (MIC)

The MIC for the studied bacterial strains, when treated with *C. macrocarpa* EO, was 0.17 mg/ml for both *S. saprophyticus* and *L. fusiformis*, 0.06 mg/ml for *B. cereus*, 1.5 mg/ml for both *K. rhizophila* and *S. liquefaciens*. The MIC of *S. terebinthifolius* to *B. cereus* and *K. rhizophila* was 2.0 mg/ml, whereas the MICs of *S. saprophyticus* and *L. fusiformis* were 0.68 mg/ml. All investigated bacterial strains showed a similar MIC value for *E. citriodora*, 0.2 mg/ml. The MIC of *S. liquefaciens* to SCHEO was 2.0 and 1.77 mg/ml for EUEO (Table 8). No antibacterial effect was observed on the tested bacterial strains at the higher concentration of tween 80 (1% v/v).

The potential antibacterial activity of the essential oils

C. macrocarpa EO showed a bactericidal effect against *S. saprophyticus* and *S. liquefaciens* as they were completely killed after treatment and failed to grow again While, for the tested *B. cereus*, the effect

Table (6): Phytochemical composition of essential oils extracted from *Eucalyptus citriodora* using Gas Chromatography/Mass Spectrometry (GC/MS) Analysis.

Peak No.	Compound name	Chemical classification	Retention time (RT, min)	Molecular Formula	Molecular weight	Area %
1	α-thujene	Monoterpene	8.031	$C_{10}H_{16}$	136	0.88
2	α-pinene	Monoterpene	8.197	$C_{10}H_{16}$	136	1.46
3	β-pinene	Monoterpene	9.776	$C_{10}H_{16}$	136	0.61
4	α-phellandrene	Monoterpene	10.119	$C_{10}H_{16}$	136	13.5
5	o-cymene	Monoterpene	10.577	$C_{10}H_{14}$	134	6.09
6	sabinene	Monoterpene	10.8	$C_{10}H_{16}$	136	24.24
7	limonene	Monoterpene	10.846	$C_{10}H_{16}$	136	1.76
8	eucalyptol	Oxygenated monoterpene	10.932	$C_{10}H_{18}O$	154	1.82
9	linalool	Oxygenated monoterpene	12.883	$C_{10}H_{18}O$	154	3.57
10	2-cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	Oxygenated monoterpene	13.495	$C_{10}H_{18}O$	154	1.49
11	2-cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	Oxygenated monoterpene	13.976	$C_{10}H_{18}O$	154	1.14
12	Cryptone	Oxygenated monoterpene	14.937	C9H14O	138	1.01
13	terpinen-4-ol	Oxygenated monoterpene	15.04	$C_{10}H_{18}O$	154	4.53
14	α-terpineol	Oxygenated monoterpene	15.372	$C_{10}H_{18}O$	154	2.56
15	2-cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, cis-	Oxygenated monoterpene	15.904	$C_{10}H_{18}O$	154	0.55
16	(1r,2s,4s,5r,7r)-5-isopropyl-1-methyl-3,8-dioxatricyclo [5.1.0.02,4]octane	Oxygenated monoterpene	16.648	$C_{10}H_{16}O$	168	2.87
17	caryophyllene	Sesquiterpene	21.769	$C_{15}H_{24}$	204	1.38
18	germacrene b	Sesquiterpene	23.606	$C_{15}H_{24}$	204	1.66
19	Guaiol	Oxygenated sesquiterpene	24.722	$C_{15}H_{26}O$	222	2.23
20	(-)-spathulenol	Oxygenated sesquiterpene	25.363	$C_{15}H_{24}O$	220	6.41
21	(1r,7s,e)-7-isopropyl-4,10-dimethylenecyclodec-5-enol	Oxygenated sesquiterpene	25.477	$C_{15}H_{24}O$	220	3.93
22	longifolene	Sesquiterpene	25.603	$C_{15}H_{24}$	204	1.56
23	14-hydroxycaryophyllene	Oxygenated sesquiterpene	25.826	$C_{15}H_{24}O$	220	0.75
24	α-eudesmol	Oxygenated sesquiterpene	26.633	$C_{15}H_{26}O$	222	7.03
25	β -eudesmol	Oxygenated sesquiterpene	26.976	$C_{15}H_{26}O$	222	3.16
26	γ- eudesmol	Oxygenated sesquiterpene	27.097	$C_{15}H_{26}O$	222	3.83
Total identified						**100.00**

Table (7): Antibacterial activity of the extracted essential oils verses three standard traditional antibiotics (Penicillin, Amoxicillin, and Ampicillin).

Tested materials	Tested Bacteria				
	B. cereus	***K. rhizophila***	***S. saprophyticus***	***L. fusiformis***	***S. liquefaciens***
Eesstinital Oil[†]					
C. macrocarpa	55.70 ± 1.2	17.50 ± 0.74	22.70 ± 1.20	31.97 ± 0.55	17.3 ± 0.88
S. terebinthifolius	13.77 ± 0.6	23.40 ± 1.32	24.97 ± 0.84	23.4 ± 0.17	13.3 ± 0.33
E. citriodora	32.50 ± 0.9	31.90 ± 1.62	25.50 ± 0.76	22.8 ± 1.01	18.3 ± 1.20
Standard Antibiotic[††]					
Penicillin	0.00 ± 0	27.70 ± 0.33	30.00 ± 0.00	30.00 ± 0.00	0 .00 ± 0.00
Amoxicillin	0.00 ± 0	38.00 ± 0	36.60 ± 0.82	40.00 ± 0.00	23.00 ± 0.00
Ampicillin	0.00 ± 0	10.00 ± 0	11.30 ± 0.67	13.00 ± 0.00	0.00 ± 0.00

[†] Essitinal oil extracted from selected three medicinal plants; [††], Standard traditional antibiotic used as a reference.

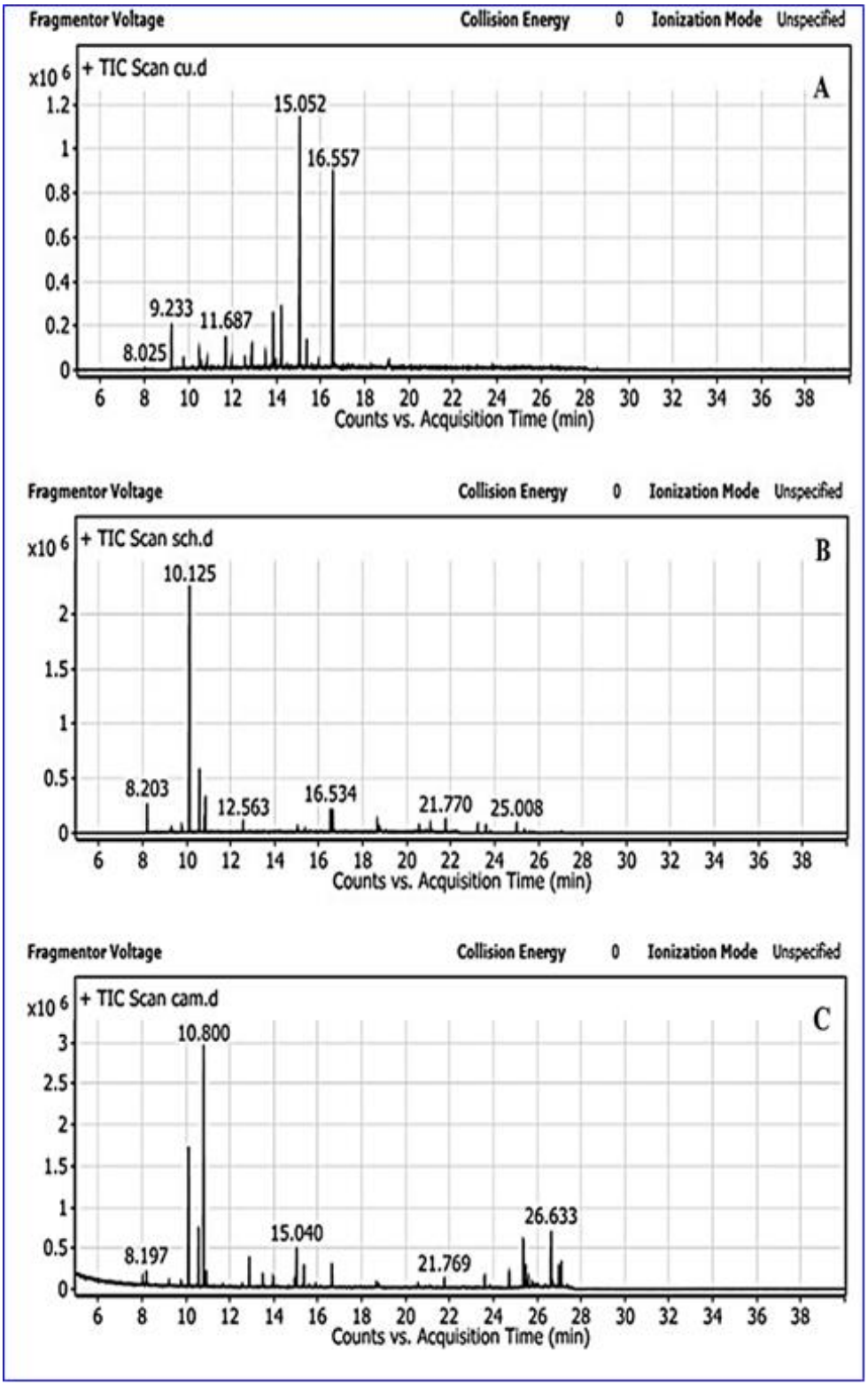

Figure (4): GC/MS Chromatogram of the essential oils extracted from different medicinal plants. A, *C. Macrocarpa*; B, *S. terebinthifolius*, and C, *E. citriodora.*

Table (8): Minimal inhibition concentration (mg ml^{-1}) assed for essential oil extracted from different selected medicinal plants against foodborne bacteria isolated from spoiled food.

Plant essential oil extracted	Isolated foodborne pathogens				
	B. cereus	***S. saprophyticus***	***L. fusiformis***	***K. rhizophila***	***S. liquefaciens***
	Minimal Inhibition conc. (mg ml^{-1})				
C. macrocarpa	0.06	0.17	0.17	1.50	1.50
S. terebinthifolius	2.00	0.68	0.68	2.00	2.00
E. citriodora	0.20	0.20	0.20	0.20	1.77

of *C. macrocarpa* oil was bacteriostatic as it lost 95.9% from its normal growth. Treatment of *K. rhizophila* and *L. fusiformis* showed that *K. rhizophila* lost 98.58% of its normal growth, and *L. fusiformis* lost 97.85% of its normal growth, meaning the effect was static against them.

The activity of *S. terebinthifolius* was bacteriostatic on the tested Gram-positive bacteria. *K. rhizophila* lost 98.7 % of its normal growth, *B. cereus* lost 94% of its normal growth, and *L. fusiformis* lost 99% % of its normal growth. *S. saprophyticus* lost 98.57% of its normal growth. The bacteriostatic activity was observed on the tested Gram-positive bacteria when treated with *E. citriodora* and allowed to grow again. *K. rhizophila* lost 91.6 % of its normal growth, *B. cereus* lost 94.5% of its normal growth, *L. fusiformis* lost 97.20 % of its normal growth, *L. fusiformis* lost 97.20 % of its normal growth, and *S. saprophyticus* lost 85.20% of its normal growth. *S. terebinthifolius* and *E. citriodora* showed a bacteriostatic effect on *S. liquefaciens* as it lost 96 and 98.3%, respectively, from its normal growth after being treated compared with its normal growth without treatment by oils.

DISCUSSION

This study studied the antibacterial activity of essential oils from some medicinal plants against some foodborne bacteria to be used as alternative natural food preservatives. Using GC/MS analysis, the essential oil of *C. macrocarpa* contained nineteen bioactive compounds. Terpinen-4-ol (32.37%), citronellol (29.29%), isopulegol (7.06%), camphor (6.21 %), and sabinene (4.31%) constitute the majority of the oil. Badawy and Abdelgaleil, (2014) reported that the major component in the essential oil of Egyptian *C. macrocarpa* leaves was terpinen-4-ol and represented by 20.29%. Also, it was reported that α-terpineol (19.01%) was the major constituent in *C. macrocarpa* (Saad *et al.,* 2017), while Salem *et al.* (2018) reported that the main constituents of the EO obtained from Egyptian *Cupressus macrocarpa* were terpinen-4-ol (23.7%), α-phellandrene (19.2%), and citronellol (17.3%). The major compounds identified in *S. terebinthifolius* were α-phellandrene (44.35%), o-cymene (10.42%), and limonene (6.44%). The chemical composition of *S. terebinthifolius* was studied in the literature by Santana *et al.,* (2012), who reported that β-longipinene (8.1%), germacrene D (23.8%), bicyclo germacrene (15.0%) and the monoterpenes α-pinene (5.7%) and β-pinene (9.1%) were the main components of the essential oil extracted from leaves of *S. terebinthhifolius*. Belhoussaine *et al.,* (2022) reported that the majority of the compounds present in the essential oil of *S. terebinthifolius* leaves were limonene 23.22%, spathulenol 14.34%, γ-terpinene 9.45%, and β-ocimene 13.32%. For *Eucalyptus citriodora* EO, Sabinene (24.24%), α-phellandrene (13.5%), α-eudesmol (7.03%), (-)-Spathulenol (6.41%), and o-Cymene (6.09%) were found to be the major constituents in *E. citriodora*. Salem *et al.,* (2018) reported that α-citronellal (56%) was the primary constituent of *E. citriodora* EO. The variation in chemical composition between the same plant species is attributed to different factors. These factors were cultivation type, vegetative stage, plant season (Ghasemzadeh *et al*., 2016), extraction method, harvesting time, plant part (Mesomo *et al.,* 2013) and age of plant has great effect on its essential oil biological activity and chemical composition (Farias *et al.,* 2023)

C. macrocarpa EO exhibited antibacterial activity against all the tested bacterial strains, with MIC values ranging from 0.06 to 1.5 mg/ml. *Bacillus cereus* showed the highest inhibition zone of 55.7 mm, with MIC of 0.06 mg/ml. According to Salem *et al.,* (2018), *Staphylococcus aureus* and *Bacillus cereus* were both inhibited by the EO from *C. macrocarpa* with MICs of 0.31 and 0.12 mg/ml, respectively, and the bactericidal effect was at 0.2 and 0.41 mg/ml respectively. Due to variations in the essential oil's chemical composition and its bioactive components, as mentioned before, the results varied.

The component of *C. macrocarpa* essential oil showed an antibacterial effect, e.g., α-phellandrene (Zhang *et al.,* 2017), camphor and limonene (Han *et al.,* 2019), γ-terpinene (Giweli *et al.,* 2012). Terpenoids are biologically active compounds with

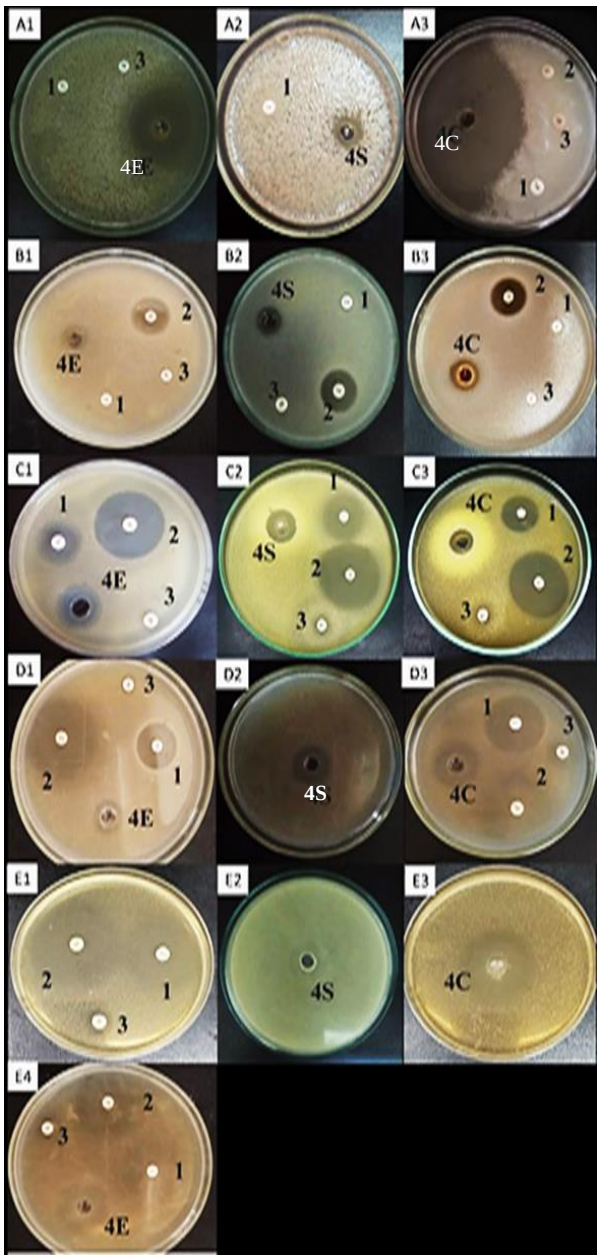

Figure (5): The antibacterial activity of essential oil extracted from tested plants which included *C. macrocarpa* (4C), *S. terebinthifolius* (4S), and *E. citriodora* (4E). Standard antibiotics represented by 1, 2, and 3 for Penicillin, Amoxicillin, and Ampicillin, respectively, were used for comparison. The antibacterial activity was assessed against various isolated bacterial strains, namely *B. cereus* (A), *S. liquefaciens* (B), *K. rhizophila* (C), *S. saprophyticus* (D), and *L. fusiformis* (E). The inhibitory zone diameters (mm) were measured compared to the tested antibiotics.

higher effects on the resistance to pathogens (Sharma *et al.,* 2019). Zhang *et al.,* (2018) reported that terpinen-4-ol showed antibacterial activity against *S. aureus* and *Streptococcus agalactiae*. Huang *et al.,* (2021) reported that terpinen-4-ol, α-terpineol, and δ-terpineol the three isomeric terpineols isolated from *Cinnamomum longepaniculatum* leaf oil by rupturing the cell wall and membrane of bacteria and resulting in cell death, have the potential to be used as antibacterial compounds. Monoterpenoids interfere with micro-organisms' physiological and biochemical processes to develop and multiply (Pandey *et al.*, 2016).

The essential oil from *S. terebinthifolius* (SCHEO) showed antibacterial activity. *S. saprophyticus* showed the highest inhibition zone diameter of 24.97 mm, but low activity was observed against the Gram-negative isolate, *S. liquefaciens* showed an inhibition zone diameter of 13.3mm, and this agrees with that reported by El-Massry *et al.* (2009) as the Egyptian *S. terebinthifolius* showed inhibitory activity against the tested, bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Low activity was observed against *E. coli*, and this may be due to lipopolysaccharides in the outer membrane of the Gram-negative bacteria, which make them resistant to external agents such as antibiotics, detergents, and hydrophilic dyes (Negi and Jayaprakasha, 2003). Gonzalez *et al.* (2004) reported that α-phellandrene and limonene were the main components of *Schinus molle* EO, which showed high activity against *Bacillus cereus.* Several EOs contain the very common cyclic monoterpene α-phellandrene, which exhibits a wide range of biological activity (Radice *et al.,* 2022). The effectiveness and reduction of any pathogenic microorganism's resistance evolution are due to the synergism between the aromatic plant components (Elshafie *et al.,* 2022). From our results, the investigated Gram-positive bacteria were found to be more sensitive to *Eucalyptus* essential oil than the tested Gram-negative. The essential oil derived from *E. citriodora* has antimicrobial properties (Salem *et al.,* 2018; Barbosa *et al.,* 2016).

From our findings, SCHEO exhibited antibacterial activity against the tested bacteria with a MIC value of 0.68 mg/ml for *L. fusiformis* and *S. saprophyticus*, but it was 2.0 mg/ml for the other tested bacteria. According to El-Massry *et al.*, (2009), SCHEO showed antibacterial activity against *Staphylococcus aureus*, *Pseudoonas aeruginosa*, and *Escherichia coli* with MIC 0.80, 0.80, and 1.10 mg/ml. For *Eucalyptus* oil, The MIC for Gram-positive bacteria (0.20 mg/ml) was lower than that for Gram-negative bacteria (1.77 mg/ml). Salem *et al.* (2018) reported that EO from *E. citriodora* leaves showed antibacterial activity with MIC values ranging from 0.06 to 0.20 mg/ml.

The antibacterial effects of natural agents may be related to some reasons, such as the inhibition of biofilm formation (Latifah-Munirah *et al.,* 2015; Molina Bertrán, 2022), the rupture of the cell wall and the cell membrane (Cui *et al.,* 2018; Wongsawan *et al.,* 2019), the influence on DNA replication (Rajkowska *et al.,* 2017). Essential oils' structure, functional groups, and composition affect their antibacterial properties (Gunasena *et al.,* 2022). Due to EOs' high concentration of lipophilic compounds, which allowed them to adhere to bacterial cell wall surfaces and membrane structures and damage their integrity, cell lysis resulted (Brozyna *et al.,* 2021). Because of their low molecular weight and highly lipophilic nature, terpenes and terpenoids can disrupt cell membranes, kill cells, or prevent the germination and sporulation of fungi that cause food spoilage. This property may be the source of an essential oil's antimicrobial activity. A previous study of *in vitro* investigations suggests that terpenes

and terpenoids perform inadequately as antimicrobials when used separately from the whole EO (Tian *et al.*, 2011). Compared to Gram-positive bacteria, Gram-negative bacteria are more resistant to hydrophobic antibiotics as Gram-negative bacteria have a surface that acts as a barrier to the entrance of macromolecules and hydrophobic substances into the bacteria cell membrane (Teerarak and Laosinwattana, 2019) as it has hydrophilic lipopolysaccharides (LPS) on their outer membranes, which acting as a barrier to hydrophobic substances, causing them to have a higher tolerance for hydrophobic antimicrobial compounds such those found in essential oils (Nikaido, 2003) and that's agreed with our results.

There have been reports that terpenes and terpenoid compounds have bactericidal properties. Antimicrobial substances must penetrate or rupture the bacterial plasma membrane to kill bacteria. Any tiniest change to the cell membrane's stability and structural integrity could harm the bacteria's regular metabolic processes, which would reduce the viability of the cell (Patra *et al.*, 2015). Terpenes, when interacting with cells, affect several metabolic processes, particularly those associated with energy production and cell membrane transport mechanisms, as reported by Aleksic and Knezevic (2014). According to several studies, most essential oil bioactive components kill bacteria by rupturing their cell walls and membranes (Diao *et al*., 2014; Xu *et al.*, 2017). The active compounds in essential oils could prevent the synthesis of nucleic acids, proteins, and other macromolecular compounds that would inhibit bacterial growth (Diao *et al.*, 2014). Essential oils can interfere with pH equilibrium, homeostasis, and the integrity of the cell membrane of microorganisms (Lambert *et al.*, 2001). Additionally, they can affect the depolarization of cell membranes (Memar *et al.*, 2017) and interfere with their permeability (Kumar *et al.*, 2019).

CONCLUSION

In conclusion, the extracted essential oils from *C. macrocarpa*, *S. terebinthifolius*, and *E. citriodora* have demonstrated remarkable potential as natural antimicrobial agents. Through rigorous experimentation, these essential oils have shown significant antibacterial properties against *S. saprophyticus*, *B. cereus*, *L. fusiformis*, *K. rhizophila*, and *S. liquefaciens*, which are common contaminants and spoilage agents in food products. The emergence of these essential oils as effective bio-preservatives presents a promising avenue for natural alternatives to traditional synthetic food preservatives. By connecting the power of these botanical treasures, it becomes possible to enhance the shelf life of food products while reducing reliance on artificial additives. However, further research is warranted to explore the full potential of these essential oils and their application in the food industry. Factors such as optimal concentrations, formulation techniques, and compatibility with various food matrices should be thoroughly investigated to ensure their safe and effective utilization. This study contributes to our understanding of the antimicrobial properties of essential oils, providing a foundation for a greener and more sustainable approach to food preservation. By utilizing these natural resources, we can strive towards a healthier and safer food industry.

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فاعلية الزيوت العطرية لثلاثة نباتات طبية كمضادات لبعض البكتريا المنقولة بواسطة الغذاء

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الملخـص العربي

تم استخلاص الزيوت العطرية من ثلاث نباتات طبيه وهي السرو الليموني- الفلفل البرازيلي والكافور الليموني (*Cupressus macrocarpa, Schinus terebinthifolius, Eucalyptus citriodora*) باستخدام التقطير المائي وجهاز كليفينجر وذلك بهدف دراسة النشاط المضاد لبعض انواع البكتريا المنقولة بواسطة الغذاء.كذلك تطويرلدراسة و التي تسبب فساد الاغذية ومن اجل تطوير مضادات للبكتريا بعد ظهور مشكلة مقاومة البكتريا للمضادات الحيوية. وقد تم التعرف باستخدام GC/MS علي 19 مركبا نشطا بيولوجيا من الزيت العطري المستخلص من نبات السرو الليموني، *Cupressus macrocarpa*، وكان Terpinen-4-ol هوالمكون الرئيسي بنسبة 32.37% ويليه مركب Citronellol بنسبة 29.29% وكان مركب α-phellandrene (44.35) ومركب O-Cymene (10.42%) هما المكونان الاساسيان للزيت المستخلص من نبات الفلفل البرازيلي، *S. terebinthifolius* ، بينما كان Sabinene (24.24%) هو المكون الاساسي للزيت المستخلص من نبات الكافور الليموني، *E. citriodora*، اظهرت الزيوت العطرية تحت الاختبار نشاط مضاد للبكتريا بمستويات مختلفة و كانت *B. cereus* الاكثر تاثرا بزيت *C. macrocarpa* وكان قطر منطقة التثبيط 55.7 مم حيث كان اقل تركيز مثبط هو (0.06جم/ مل) كما اظهرت مقاومة ضد المضادات الحيوية المختبرة (البنسلين والاموكسيسيلين والامبيسيللين) . *اظهرت البكتريا سالبة جرام S.liquefaciens* مقاومة ضد البنسلين و الامبيسيلين بينما تم تثبيط النمو من خلال الزيوت الاساسية بدرجات مختلفة حيث كان اقل تركيز للزيت *C. macrocarpa* هو 1.5مجم/مل بينما 2مجم/مل. لزيت*S.terebinthifolius S* وكان 1.77 مجم/مل لزيت *E. citriodora* وقد اظهر تأثير مميت دائم ضد *S. saprophyticus* و *S. liquefaciens* بينما كان التاثير مثبط مؤقت ضد*L. fusiformis,* و *K.rhizophila*و *B. cereus*. اظهرت الزيوت المستخلصة من كل من نبات *S. terebinthifolius* و*E. Citriodora* تأثير مثبط مؤقت ضد البكتريا تحت الاختبار. النتائج المتحصل عليها سوف تفيد في التطبيق العملي للزيوت العطرية المستخلصة كمضاد للبكتريا المقاومة للمضادات الحيوية وكمادة حافظة للغذاء.